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TECHNICAL MANUSCRIPT 22

**EXPERIMENTAL PRIMARY
CUTANEOUS COCCIDIOIDOMYCOSIS
IN THE MONKEY**

NOVEMBER 1962

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ABSTRACT

Primary cutaneous coccidioidomycosis was studied in the monkeys (Macaca mulatta) to find a suitable strain of Coccidioides immitis for use as a viable vaccine. Intradermal inoculation (medial surface of the right forearm) produced more severe vaccination reactions (draining vaccination site and axillary lymph node hypertrophy) than subcutaneous injection. A subcutaneous vaccine dose of ten arthrospores resulted in less reaction than a 100-spore dose. Moreover, dissemination beyond the regional lymph nodes did not occur, following injection of ten spores of even the most virulent strains of C. immitis. Two of the five strains tested (Silveira, M-11, D-76; Cash, and a colonial isolate from Cash designated as CW1) exhibited very mild vaccination reactions and appeared to have been cleared from the tissues upon necropsy at 10 months post-challenge. These two strains (Cash and CW1) appear promising for further immunological studies with a viable vaccine.

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I. INTRODUCTION

Studies on experimental primary cutaneous coccidioidomycosis were initiated with the objective of developing a viable vaccine against the pulmonary form of the disease. Smith's¹ epidemiological evidence of the lack of second infections in thousands of patients with a diagnosis of primary pulmonary coccidioidomycosis, and the lack of evidence of systemic dissemination in approximately 18 proved cases of primary cutaneous infection²⁻⁴, have indicated the feasibility of such a vaccine.

The authors, in 1961⁵, reported that the subcutaneous inoculation of Coccidioides immitis in the forearm of monkeys (Macaca mulatta) protected them from a later respiratory challenge with the same organism. It was also shown that as little as ten arthrospores gave this protection without dissemination beyond the axillary lymph nodes, and that the protection was not strain specific.

The present report covers further studies of cutaneous coccidioidomycosis using five C. immitis strains of graded virulence in man and in various experimental animals. The objective of the present study was ~~threefold: to find a strain that would be used safely as a viable vaccine~~ (i.e., no dissemination beyond the regional lymph nodes); to find one that would exhibit less severe side-effects (i.e., mild vaccination reaction, and less involvement of the axillary lymph nodes); and to compare the effects of subcutaneous and intradermal inoculation of the organism.

NOTE: The animals used in this study were maintained in compliance with the "Principles of Animal Care" as promulgated by the National Society for Medical Research, Bio-medical Purview, 1:14, 1961.

II. METHODS

Rhesus monkeys, in groups of four, were inoculated either subcutaneously or intradermally in the medial surface of the right forearm, with either 10 or 100 viable arthrospores of C. immitis, strains Silveira, Cash, M-11, D-76, or CW1 (isolate of Cash with reduced virulence and altered colonial morphology).

The monkeys were observed for a period of ten months for clinical signs of disease and for gross evidence of tissue reaction to the injections. At ten months, all animals were sacrificed (pentobarbital sodium) for complete necropsies. Precipitin titers were obtained on sera obtained at terminal bleeding, and cultures were made routinely from the inoculation site, the right axillary lymph node, and the lung. Any suspected lesions in other organs or tissues were also cultured.

Histopathological studies were made on sections of the inoculation site, the right axillary lymph node, and all the body organs, using the Giemsa as well as specific fungus stains. Lung sections were also checked with fluorescent antibody techniques.

III. RESULTS

The results of this study are shown in the following tables:

A. SUBCUTANEOUS VERSUS INTRADERMAL INOCULATION (Table I)

Intradermal vaccination with viable *C. immitis* produced twice as many open and draining lesions at the site of inoculation than subcutaneous vaccination. Moreover, small multiple skin lesions developed progressively, from the vaccination site to the axillary lymph nodes, similar in appearance to those formed in Sporotrichosis infections.

Approximately an equal number of monkeys exhibited involvement of the axillary lymph nodes in both groups, but this condition was first noted by the fourth week in the intradermal vaccinees, compared with the ninth week in the subcutaneous vaccinees. For these reasons, subcutaneous vaccination was chosen as the route of inoculation for further studies.

TABLE I. TISSUE RESPONSE: ROUTE OF INOCULATION

Weeks	Subcutaneous		Intradermal	
	Draining Lesions	Enlarged Lymph Nodes	Draining Lesions ^a	Enlarged Lymph Nodes
4	0/8	0/8	3/8	2/8
6	2/7	0/7	4/8	3/8
7	0/7	0/7	4/8	4/8
9	0/7	2/7	5/8	4/8
21	1/7	2/7	0/8	3/8
Total Incidence of Response	3/7	4/7	7/8	4/8
Total Inoculated	(43%)	(57%)	(88%)	(50%)

a. Multiple (vaccination site to axillary lymph node).

B. VACCINATION DOSE LEVEL (Table II)

A subcutaneous dose level of 10 or 100 viable organisms produced equal numbers of open lesions at the site of inoculation; however, the higher dose resulted in six times as many visibly enlarged lymph nodes as were noted in the ten-spore group. This lymph node involvement was first noted at six weeks and at 21 weeks, respectively, in the high and low dose groups.

TABLE II. TISSUE RESPONSE: DOSE LEVEL
(Subcutaneous Inoculation)

Weeks	10		100	
	Draining Lesions	Enlarged Lymph Nodes	Draining Lesions	Enlarged Lymph Nodes
4	0/12	0/12	1/12	0/12
6	1/12	0/12	2/11	2/11
7	0/12	0/12	1/11	2/11
9	1/12	0/12	1/11	4/11
21	1/12	1/12	0/11	2/11
Total Incidence of Response	3/12	1/12	3/11	5/11
Total Inoculated	(25%)	(8%)	(27%)	(46%)

C. COMPARISON OF STRAINS (Tables III and IV)

Subcutaneous inoculation of *C. immitis*, strains Silveira, M-11, or Cash, resulted in significant differences in tissue response and serological response to presence of the organism. Open lesions at the site of inoculation and visibly enlarged axillary lymph nodes were noted in approximately one-half of the monkeys inoculated with strain Silveira and one-quarter of those receiving strain M-11. These conditions were not evident in the group inoculated with strain Cash.

Precipitin titers varying from 1:8 to 1:512 were noted at ten months in animals vaccinated with strain Silveira; negative to $\pm 1:128$ with strain M-11; and all negative with strain Cash. As expected, the higher dose levels produced higher titers. The absence of a titer in some of these animals does not indicate lack of protection, because neither the precipitin nor the complement-fixation level is a measure of immunity (a positive skin reaction to the intradermal injection of coccidioidin was noted in all animals at 16 weeks post-inoculation).

**TABLE III. TISSUE RESPONSE: STRAINS
(Subcutaneous Inoculation)**

Weeks	Silveira			M-11			Cash		
	Draining Lesions	Enlarged Lymph Nodes		Draining Lesions	Enlarged Lymph Nodes		Draining Lesions	Enlarged Lymph Nodes	
4	0/8	0/8		1/8	0/8		0/6	0/6	
6	2/7	0/7		1/8	2/8		0/6	0/6	
7	0/7	0/7		1/8	2/8		0/6	0/6	
9	0/7	2/7		2/8	2/8		0/6	0/6	
21	1/7	2/7		0/8	1/8		0/6	0/6	
Total Incidence of Response	3/7 (44%)	4/7 (57%)		2/8 (25%)	2/8 (25%)		0/6 (0%)	0/6 (0%)	
			Total Inoculated						

TABLE IV. SEROLOGY

Strain	Dose	Precipitin Titer ^a /	
Silveira	10	1:8	±1:64
		1:8	±1:64
	100	±1:128 ±1:128	1:512 ^b / N.T. ^c /
M-11	10	±1:32 Negative	Negative ±1:32
	100	±1:128 Negative	Negative ±1:128
Cash	10	Negative Negative	Negative
	100	Negative Negative	Negative

a. Titers of individual animals at ten months.

b. Disseminated.

c. Not tested (early death-disseminated).

D. HISTOPATHOLOGICAL STUDIES (Tables V and VI)

Upon necropsy of the 100-spore group at ten months, the highest incidence of residual organisms was noted in the tissues of animals inoculated with the Silveira strain and in decreasing order in the M-11 and Cash strains. Three of the four animals receiving strain Silveira exhibited systemic dissemination beyond the axillary lymph node; one of the M-11 vaccinee group showed dissemination of a minimal amount. It is important to note that those injected with strain Cash remained free of dissemination even at this high dose.

A similar picture was exhibited by animals receiving the 10-spore dose, with two important exceptions. No systemic dissemination occurred with any of the strains at this dose level; and no residual organisms were found at the skin site or in the regional lymph nodes of any of the animals inoculated with strain Cash. In approximately 98 per cent of the incidences, the presence of residual organisms in the tissues was corroborated with positive cultures.

TABLE V. HISTOPATHOLOGY: 100-SPORE DOSE
(Organisms Present)

Strain	Vaccination Site	Right Axillary Lymph Node	Lung	Other
Silveira	+	+	-	+ Liver
	+	-	-	-
	+	+	+	+ Liver, spleen, kidney, adrenal glands
	+	+	+	+ Liver, inguinal lymph node, thoracic abscess
M-11	-	NE ^a /	-	-
	+	+	-	+ Left axillary lymph node
	+	+	-	-
	-	+	-	-
Cash	-	-	-	-
	-	+	-	-
	-	-	-	-

a. Not examined, culture positive.

TABLE VI. HISTOPATHOLOGY: 10-SPORE DOSE
(Organisms Present)

Strain	Vaccination Site	Right Axillary Lymph Node	Lung	Other
Silveira	-	+	-	-
	+	NE ^a /	-	-
	+	-	-	-
	+	+	-	-
M-11	-	-	-	-
	+	+	-	-
	-	-	-	-
	-	-	-	-
Cash	-	-	-	-
	-	-	-	-
	-	-	-	-

a. Not examined.

In summary, it can be seen (Table VII) that C. immitis, strain Cash, shows promise as a safe viable vaccine against respiratory coccidioidomycosis. Used at the 10-spore dose level (reported previously as giving equal protection to that of much higher doses) the undesirable effects from vaccination reaction would be at a minimum, and it appears that clearing of the organism from the body would occur.

Studies with the last two strains appearing in this table, D-76 and CW1, are incomplete, but preliminary results are promising with at least one of them. Strain CW1 has shown extremely low virulence in mice and appears to die out when injected into either mice or monkeys. This strain, and strain Cash, will be used for further studies of the protective value of viable vaccines against respiratory challenge with C. immitis.

TABLE VII. COMPARATIVE SUMMARY

Strain	Open Vaccination	Enlargement of Lymph Nodes	Dissemination	Residual Organisms
100-Spore Dose				
Silveira	+	+	+	+
M-11	+	+	+	+
Cash	-	-	-	+
D-76	-	+	I ^a /	I
CW1	-	-	I	I
10-Spore Dose				
Silveira	+	+	-	+
M-11	+	+	-	+
Cash	-	+	-	-
D-76	-	+	I	I
CW1	-	-	I	I

a. Incomplete data.

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